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Determination of Atrazine and Selected Respective Metabolites Using Strong Cation Exchange Resins

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DETERMINATION OF ATRAZINE AND SELECTED RESPECTIVE METABOLITES USING STRONG CATION EXCHANGE RESINS

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DETERMINATION OF ATRAZINE AND SELECTED RESPECTIVE METABOLITES USING STRONG CATION EXCHANGE RESINS

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DETERMINATION OF ATRAZINE AND SELECTED RESPECTIVE METABOLITES USING STRONG CATION EXCHANGE RESINS

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Directed by: Dr. Eric D. Conte, Dr. Stuart Burris, and Dr. Lester Pesterfield

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A new solid phase extraction procedure (SPE) for the removal of atrazine and three respective metabolites including deisopropylatrazine, desethylatrazine, and didealkylatrazine from aqueous samples is presented. Octadecyl bonded silica (C_{18}) is widely used in analytical preconcentration schemes. While C_{18} presents a facile method of analyte removal, a new approach is needed to achieve greater trapping for polar analytes. In this study strong cationic exchange (SCX) resins consisting of sulfonated polystyrene are investigated for their extraction capabilities of the selected analytes. By exploiting acid-base and pi-pi interactions the analytes are trapped and therefore extracted from aqueous samples. Salt solutions are used to remove these analytes through cation exchange from the sorbent. Percent recovery of atrazine and its metabolites was determined by high performance liquid chromatography. Comparison of SCX resins to commercially available C_{18} resin was determined to assess the benefits of this novel technique. Strong cation exchange resins investigated include SCX-2, Dowex 50WX2-400, Dowex 50WX4-400, and Dowex 50WX8-400.

I. INTRODUCTION

A. Background

Numerous techniques have been developed for the extraction of s-triazines and their degradation products. The name is derived from its heterocyclic ring structure containing three nitrogen atoms alternating with three carbon atoms in a symmetrical pattern giving rise to the name symmetrical triazine, or s-triazine. Of all of the techniques, liquid-liquid extraction (LLE), semipermeable membrane device, super-critical fluid extraction, solid phase micro extraction, and solid phase extraction (SPE), SPE is the most widely employed[1]. SPE has been further explored to optimize extraction conditions of atrazine, desethylatrazine, deisopropylatrazine, and didealkylatrazine. Several analytical techniques have been employed for trace level determination of these compounds including: gas chromatography-mass spectrometry,[11] gas chromatography-thermo ionic,[26] gas chromatography-nitrogen phosphorus,[20] micellar electrokinetic chromatography,[6] enzyme linked immunosorbent assay,[25] and high performance liquid chromatography-ultra violet visible detector.[18] In this study eluted fractions from the column containing the SCX resin were analyzed by reverse phase high performance liquid chromatography equipped with a photodiode array detector (PDA).

B. Extraction of Atrazine and Degradation Products From Aqueous Solution

The most recent and widely used extraction technique for the removal of herbicides from aqueous samples is SPE. Methods for extraction and preconcentration of contaminants have become necessary due to more rigorous limits for water purity, and SPE has become the preferred method over traditional extraction procedures because it reduces the degree of sample handling and solvent consumption. The most popular SPE

sorbent for simultaneous atrazine and metabolite extraction from aqueous samples is octadecyl bonded silica (C_{18}). In this SPE procedure, aqueous samples containing atrazine, desethylatrazine, deisopropylatrazine, and didealkylatrazine are passed through a column containing the resin. The trapped analyte is eluted with an organic rinse solution. Eluted fractions from the column are then analyzed by RP-HPLC-PDA; the for the nitrogen containing rings of s-triazines strongly absorb in the UV region 210nm – 240nm,[2] which make them easily detectible by PDA. While gas chromatography (GC) has been widely used for the determination of s-triazines and their degradation products, RP-HPLC-PDA is favored.[2] S-triazines which are highly polar, volatile, and thermally unstable, may not be directly injected into a GC. Detection of these compounds using GC requires some sample preparation before instrument injection.

C_{18} based sorbents trap analytes based on weak van der Waals forces. Atrazine contains a six membered ring with alternating carbon and nitrogen, which makes it a very polar molecule. As atrazine metabolizes it looses its alkyl side chains, which increases its polarity. Because C_{18} based sorbents rely solely on weak van der Waals forces to extract these metabolites from aqueous samples, it does not provide full removal of all compounds. Thus, a new SPE sorbent is needed. Strong cation exchange (SCX) resins have been investigated to determine optimum extraction capability.

The SCX resins used in this study consist of sulfonated polystyrenes. The sulfonic acid group associated with the resin is strongly acidic, and attracts or exchanges cationic species on the resin. The bonded functional group is charged over a wide pH range and can therefore exchange with any cation, whether it be a hard or soft cation.

This approach is consistent with conventional SPE techniques; however, it incorporates SCX resins to enhance extraction capabilities by exploiting stronger molecular interactions. Efficient and quantitative removal and recovery of analytes from aqueous samples is achieved as a function of acid-base interaction as well as cation exchange. An acid-base interaction is the driving force for analyte capture. As the analyte is introduced to the resin, it is protonated by the hydrogen functional group associated with the resin, which is highly acidic. From there, an acid base reaction occurs between the analyte and the resin, and the analyte is effectively trapped on the resin. After this trapping step, a rinse solution made of a salt, 2-propanol, and deionized water is used. The rinse solution used in this technique is environmentally benign, eliminating traditional organic solvents. It is also important to note that the resin exists initially in protonated form. The analytes' interaction with the resin is dependent upon the nature of the functional group. The protonated form of the resin allows the analyte to form a cation which is attracted to the resulting anionic resin. The introduction of a salt to the resin induces cation exchange and release of the analyte from the resin. This cation exchange is responsible for analyte recovery from the resin. This newly developed method is a model for efficient and quantitative analyte removal.

The extraction procedure is presented in Figure 1. In part A, a cation exchange resin is presented. The initial form of the resin is protonated; exchange plays an important role in analyte extraction, and recovery. The proton associated with the resin is the driving force for the trapping mechanism. In part B, a sample containing a known concentration of triazines is introduced to the column that holds the SCX resin. The analytes are trapped on the resin through an acid-base interaction. The lone pairs of electrons on the amine

nitrogens remove hydrogens from the resin, creating a positively charged analyte. The protonated analyte is strongly attracted to the deprotonated resin. In part C, the elution step, a rinse solution consisting of water and a soluble alcohol and containing an ion exchanger cation (i.e. Na^+ from $NaC_2H_3O_2$) is introduced to the column. The cation takes the place of the positively charged analyte, resulting in analyte recovery. The residual acetate anion abstracts the proton from the analyte to form acetic acid, a weak acid. The eluate is compatible with direct injection into the HPLC. Because the sample is slightly acidic, atrazine and its metabolites may still be in the protonated form upon injection into the HPLC. These cations may interact with the silanol groups on the stationary phase of the RP-HPLC, resulting in peak distortion. A buffered mobile phase is necessary to remedy this.

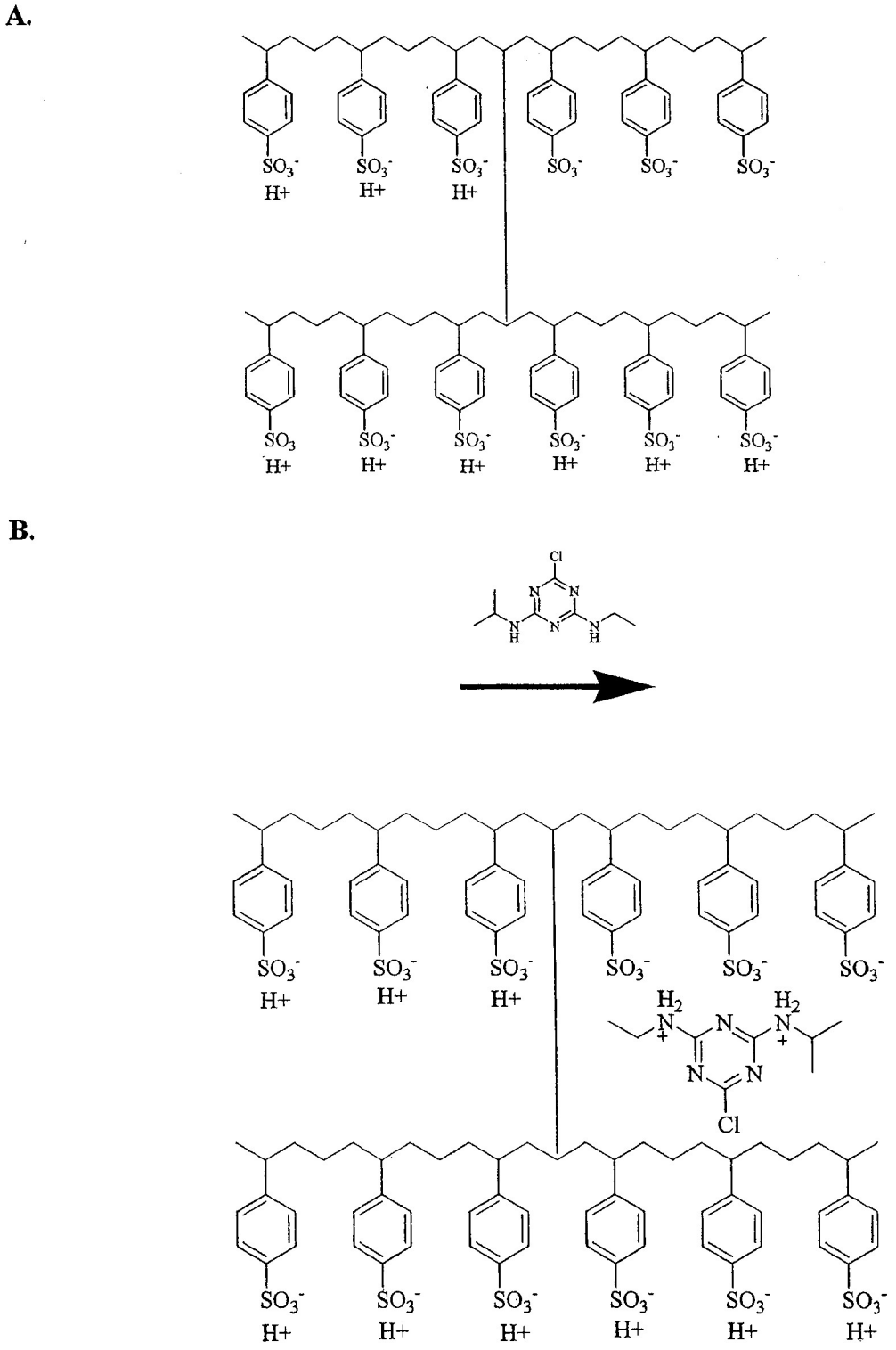


Figure 1. Strong cationic exchange resin used for solid phase extraction process
A. Initial form of strong cationic exchange resin
B. A samples containing an analyte (atrazine) is passed through a column containing the resin and adheres to the resin

C.

0.25M $NaC_2H_3O_2$
50% 2-propanol

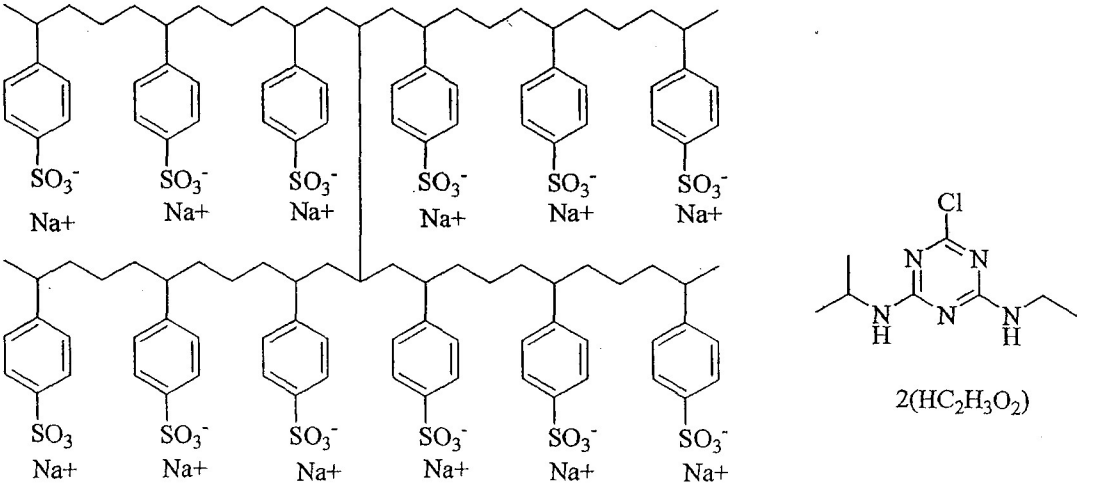


Figure 1. C. The elution step. A solution containing an exchange cation (i.e. Na^+ from $NaC_2H_3O_2$) in a mixture of water and a soluble alcohol is passed through the column containing the sorbent.

C. Atrazine

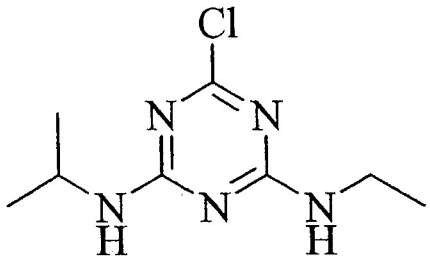
Atrazine is an s-triazine class herbicide widely employed by agriculturalists. It is used to inhibit the growth of annual broadleaf, and grassy weeds in row crops such as field corn, sorghum, and sugar cane.[11] Atrazine is often applied as a water-based spray, but may also be applied as a dry powder mixed with bulk fertilizers.[3] Regardless of application, it successfully destroys most weeds by inhibiting primary events of photosynthesis in the chloroplasts. Its ease of application and superior effectiveness, along with its low cost makes atrazine the herbicide of choice. When atrazine is applied to crops, it is absorbed into the soil and travels into the groundwater below. Atrazine begins to metabolize once it is exposed to an aqueous environment, generating several new compounds.[8] Its metabolites include desethylatrazine, desisopropylatrazine, didealkylatrazine, hydroxyatrazine, desethylhydroxyatrazine, deisopropylhydroxyatrazine, didealkylhydroxyatrazine, simazine, propazine, ametryn, prometryn, tertbutryn, hydroxy simazine, desethyl tertbutylazine, tertbutylazine, and prometon.[8] In this study we have chosen to investigate atrazine, desethylatrazine, deisopropylatrazine, and didealkylatrazine. This area of Kentucky is a karst region, meaning it has a large amount of limestone in the environment. Limestone ($CaCO_3$) acts as a natural buffer, keeping water at or around a neutral pH. The metabolites chosen are those that persist at or around a neutral pH.[3] Because groundwater is our primary source of fresh drinking water the negative health effects of atrazine have become a significant concern of the United States Environmental Protection Agency (EPA).

The EPA has set the maximum contamination limit (MCL) of atrazine for the safe consumption of drinking water at 3 parts per billion (ppb) or 3 ng/mL.[11] Above the MCL

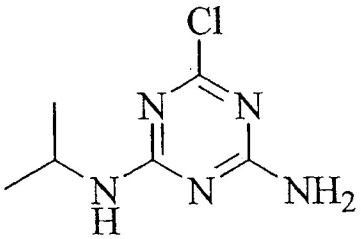
short term health effects include: congestion of heart, lungs and kidneys; low blood pressure; muscle spasms; weight loss; and damage to adrenal glands. Long-term health effects include: weight loss, cardiovascular damage, retinal and some muscle degeneration. In addition to these many effects, atrazine is also thought to be carcinogenic.[24] As atrazine and its metabolites, or degredation products, are found primarily in ground water a procedure for the determination of these compounds was investigated. This study focuses on four of the compounds including: atrazine, desethylatrazine, deisopropylatrazine, and didealkylatrazine. The structure of these compounds is shown in Figure 2, and their IUPAC names and molar masses are listed in Table 1.

D. Purpose of This Study

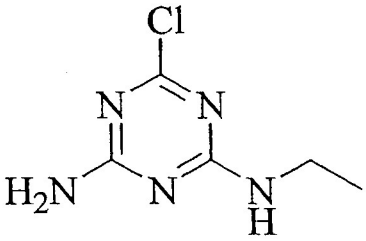
The purpose of this study is to optimize an extraction method for the removal of atrazine and selected metabolites from aqueous samples using SPE, incorporating several different strong cation exchange resins in the analytical technique. Auxiliary goals included minimization of sample handling, reduction of the amount of solvent used, employment of environmentally benign solvents that also allow direct RP-HPLC injection, and comparison of extraction capabilities of silica-based C_{18} resins to a SCX resin.



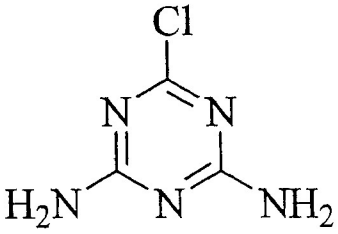
atrazine



desethylatrazine



deisopropylatrazine



didealkylatrazine

Figure 2. The structures of compounds used in this study

Table 1. Basic Parameters Of The Compounds Used In This Study.

Common Name	IUPAC Name	MW*
Atrazine	2-chloro- 4-ethylamino- 6-isopropylamino- 1,3,5-triazine	215.6851
Desethyl atrazine	2-chloro- 4-amino- 6-isopropylamino- 1,3,5-triazine	187.6315
Deisopropylatrazine	2-chloro- 4-ethylamino- 6-amino- 1,3,5-triazine	173.6047
Didealkylatrazine	2-chloro- 4,6-amino- 1,3,5-triazine	145.5511

* molecular weight - grams per mole

II. EXPERIMENTAL

A. Materials

A.1. Reagents

Atrazine (Ultra Scientific, North Kingstown, RI), desethylatrazine, deisopropylatrazine, and didealkylatrazine (Sigma-Aldrich, Milwaukee, WI) powders were used to make standard solutions. Potassium phosphate (J.T. Baker, Phillipsburgh, NJ) was used to prepare buffers. HPLC grade 2-propanol (No. A451-4) and acetonitrile (No. A998-4) were purchased from Fischer Scientific (Fair Lawn, NJ). Dowex 50WX2-400 and 50WX4-400 were purchased from Acros Organics (Fair Lawn, NJ), and Dowex 50WX8-400 was purchased from Aldrich Chemical Company, Inc. (Milwaukee, WI). Bulk Isolute Sorbent SCX-2 and Bulk Isolute Sorbent C_{18} were purchased from International Sorbent Technology Ltd. (Hengoed Mid Gladm, UK). Sodium, potassium, calcium, and ammonium acetate were purchased from Matheson, Coleman, and Bell (Norwood, OH, USA). Phosphoric acid (No. PX0995-3, Fischer Scientific, Fair Lawn, NJ) was used to adjust the pH of the HPLC mobile phase.

A.2. Apparatus

A.2.1 Varian High Performance Liquid Chromatograph (HPLC)

A Varian HPLC with a Prostar Model 910 auto sampler, Model 9012 reciprocating pump, and Model 330 photodiode array detector was used in this study. Samples were stored in glass cuvettes and injected using this fully automated system. A Microsorb C_{18} column (Varian Analytical Instruments, Walnut Creek, CA) was utilized as the stationary phase with 20 mM potassium phosphate (pH 6.8) and acetonitrile as the mobile phase.

The data collected from the HPLC was used to calculate percent recovery of atrazine, desethyl atrazine, deisopropyl atrazine, and didealkyl atrazine.

A.2.2 Adjusta-Chrom Chromatography Column

All sorbents were held in a 360 X 10 nm I.D. Adjusta-Chrom (Ace Glass, Vineland, NJ) adjustable chromatography column. In order to hold small volumes of sorbent 15 and 30 cm plastic extenders equipped with glass frits were included. The Adjusta-Chrom column helped maintain sample purity by allowing minimal contact with possible sources of contamination.

A.2.3 Soxhlet Extraction Apparatus

Each Dowex resin used in this study was cleaned over-night or no less than eight hours with 2-propanol in a Soxhlet extraction apparatus.

A.2.4 Sample Delivery System

Each 500-mL sample delivered to the sorbent through the Adjusta-Chrom column was first loaded into a 2 liter round glass bulb (No. 5824-15, Ace Glass, Louisville, KY) and pumped through the sorbent. The top of the bulb was connected to an argon tank and pressurized inside to 10 psi to force the sample through the sorbent. The pressure was controlled by a Brooks Model 8601 pressure regulator (Hatfield, PA, Model 8601).

A.2.5 Syringe Pump

Conditioning and rinse solutions were pushed through an Adjusta-Chrom column using a KDScientific Single Syringe Infusion Pump equipped with a glass syringe. The flow rate was set at 2 mL/min.

B. Procedure

B.1. Standard Preparation

The results obtained from each set of experiments were compared to a standard prepared using 500 μL of an atrazine mixture containing atrazine, desethylatrazine, deisopropylatrazine, and didealkylatrazine at 49, 44, 46, and 62 ppm, respectively. The cocktail was prepared in 50% acetonitrile and was also used to spike the aqueous samples. This volume was then diluted to 2 ml using a solution with the same percentage of 2-propanol as in the rinse solution used in the elution step.

B.2. Extraction of Atrazine and Metabolites in a Hard Water Matrix

Two milliliters of the desired sorbent was held in the Adjusta-Chrom column and conditioned with 10 mL of a 2-propanol and water solution. The 2-propanol solution contained the same percentage 2-propanol as in the elution step. The sorbent was then air dried using the syringe pump. Finally, 6 mL of deionized water was pumped through the sorbent, which was not allowed to dry. After this conditioning, step a 500 mL sample of deionized water spiked with 500 μL of 1 M $CaCl_2$ to emulate a hard water matrix, and 500 μL of the atrazine mixture, was pumped through the sorbent.

B.3. Sorbent Preparation

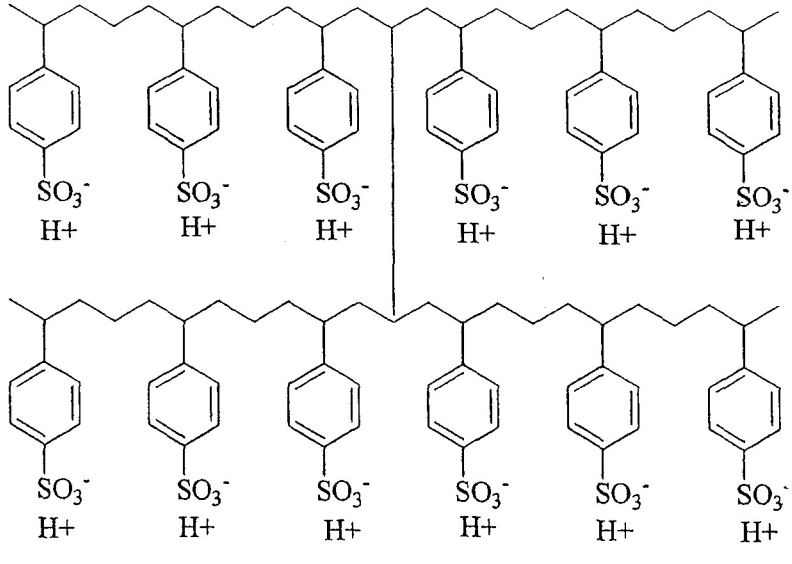
The strong cation-exchange resins were received in their hydrogen form. The Dowex resins were cleaned as described above and stored in clear glass containers with ground glass stoppers. The resins investigated in this study include: C_{18}, SCX-2, Dowex 50WX2-400, Dowex 50WX4-400, and Dowex 50WX8-400 (Table 2).

Table 2. Structures of Resins Used in this Study

C_{18}

SCX-2

Dowex Resins



C. Experimental Procedure

Each experiment was conducted in the same manner and performed in triplicate. Two milliliters of the desired sorbent was conditioned as described in section B2. The sample containing atrazine, desethyl atrazine, deisopropyl atrazine, and didealkyl atrazine was then applied to the column.

C.1. Extraction Set-Up

Each SPE sorbent was enclosed in an Adjusta-Chrom column as described in section A.2.2. The column itself was made of glass. The frits found at the ends of each of the plastic plungers were made of silica. The frits of the Adjusta-Chrom column were in direct contact with the sorbent. After each sample application the sorbent was rinsed with 2 mL of an elution solution five times. The percent recovery was determined for each rinse by comparing the results of the chromatograms of each rinse to a standard. Each 500 mL sample was held in a 2 liter round glass bulb (5824-15, Ace Glass, Louisville, KY) and pumped through the sorbent. The Adjusta-Chrom column was connected to the glass bulb using a 25mm plastic adapter (Ace Glass) connected to 1/8 inch Teflon tubing using a P-621 adapter (Scievex, Oak Harbor, WA). The top of the bulb was connected to an argon gas tank equipped with 1/8 inch Teflon tubing. Gas was pumped through the tubing to create enough pressure inside of the bulb (approximately 10 psi) to push the sample though the bottom of the bulb and up through the top of the column and finally the sorbent. The pressure exerted was controlled by a Brooks Pressure Regulator (Hatfield, PA, Model 8601). After the sample was applied, the sorbent was rinsed five times with 2 mL of a chosen rinse solution. This elution or rinse solution was delivered onto the column by pushing it through 1/8 inch Teflon tubing using a KDScientific Single

Syringe Infusion Pump equipped with a glass syringe. The flow rate was approximately 2 mL/min. For each individual rinse the percent recovery was determined by analysis on a Varian HPLC system.

C.2. Parameters of the HPLC

The gradient was programmed on a Varian Reciprocating Pump (model 9012) using acetonitrile and 20 mM potassium phosphate (K_3PO_4), at pH6.8. The gradient was programmed to start at 5:95 (v/v) acetonitrile and 20 mM potassium phosphate sustaining for five minutes, then increasing at a steady rate for and additional 15 minutes to 100:0 (v/v) acetonitrile and 20mM potassium phosphate. The gradient was performed with a constant flow rate of 1 mL/min. Each injection had a 25 μL injection volume on a Varian Prostar Autosampler (model 910).

D. Mean Recovery and Standard Deviation

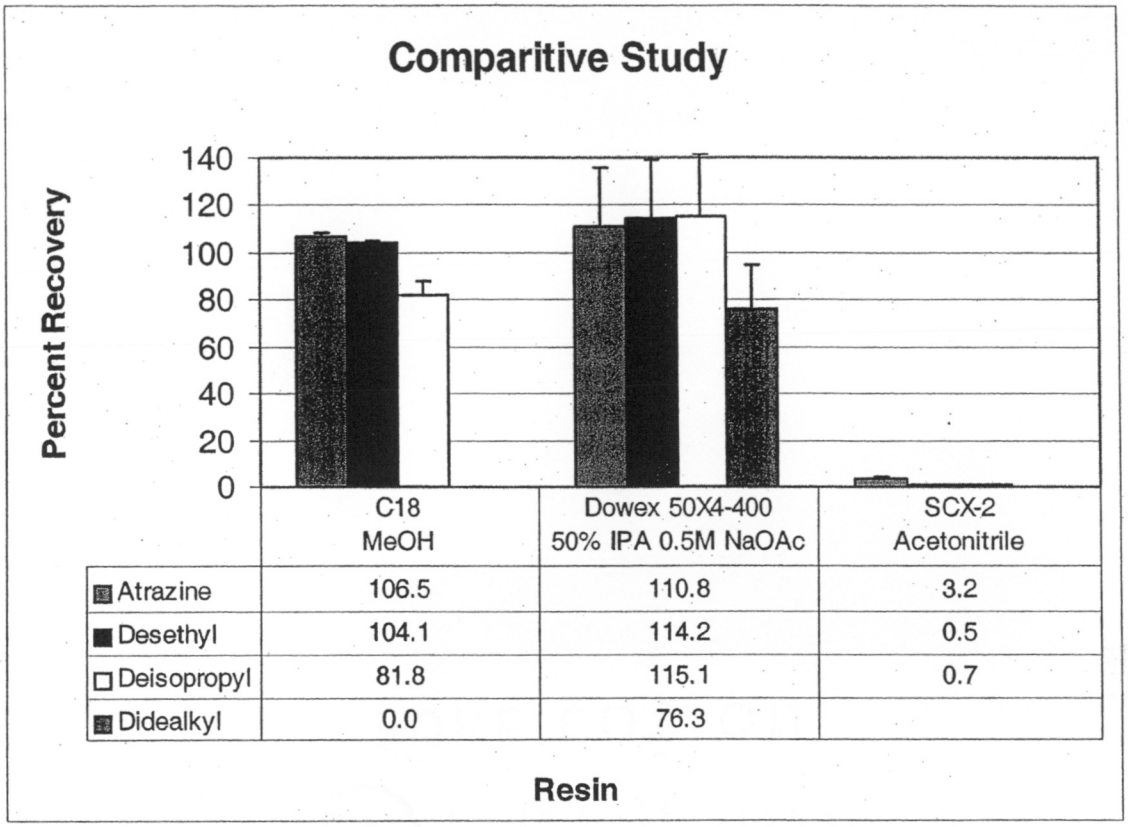
The results from each of the three trials were used to determine an average recovery for each experiment and its standard deviation. Each figure shows columns displaying errors bars, which indicate the standard deviation associated with each respective analyte.

III. RESULTS AND DISCUSSION

A. Comparative Studies

Different types of sorbents have been employed in SPE techniques to extract atrazine and its metabolites from aqueous samples. One of the most widely used sorbents is silica based C_{18}.[1] While successful trapping of atrazine is achieved this resin is unable to achieve complete recovery of more polar metabolites of atrazine, specifically didealkyl atrazine and deisopropyl atrazine. In this study we have investigated the trapping capabilities of the more polar metabolites of atrazine using strong cation exchange resins. Compared to conventional silica based C_{18} resins, strong cation exchange resins allow multiple intermolecular interactions. Silica based C_{18} is composed of alkyl chains supported on a silica particle. This type of resin traps analytes based upon weak van der Waals forces. Recovery for atrazine is sufficient, however, more polar metabolites such as didealkyl atrazine, and deisopropyl atrazine are not fully recovered. By incorporating different strong cation exchange resins into our procedure we may take advantage of those intermolecular forces, which allow greater trapping of more polar metabolites. The first strong cation exchange resin investigated was SCX-2 (Table 2). This type of resin allows van der Waals interactions, and acid-base interaction due to the hydrogen accompanied by the SO_3^- group. The second type of resin we investigated for this comparative study was Dowex 50WX4-400. This resin is a sulfonated polystyrene based resin, which is initially in a hydrogen form. This type of resin allows several intermolecular interactions such as: pi-pi stacking, van der Waals interactions, acid-base interactions, and cation exchange. Upon investigation of these three resins, data showed the silica based C_{18} resin offered poor recovery for more polar metabolites, while Dowex

50WX4-400 showed recovery for all metabolites, including those of greater polarity, including didealkyl atrazine and deisopropyl atrazine Figure 3. Although recovery using Dowex 50WX4-400 was not complete for all analytes, experiments using this resin were optimized. These findings led to further investigate a series of Dowex resins including: Dowex 50WX2-400, Dowex 50WX4-400, and Dowex 50WX8-400.



	C18 MeOH	Dowex 50X4-400 50% IPA 0.5M NaOAc	SCX-2 Acetonitrile
Atrazine	106.5	110.8	3.2
Desethyl	104.1	114.2	0.5
Deisopropyl	81.8	115.1	0.7
Didealkyl	0.0	76.3	

Figure 3. A comparative study showing the percent recovery of atrazine, desethyl atrazine, deisopropyl atrazine, and didealkyl atrazine using different resins including: silica based C_{18}, Dowex 50WX4-400, and SCX-2. The rinse solutions for each of the columns were: methanol, 0.50M NaOAc in 50% IPA, and 50% IPA respectively.

B. Dowex Resins

Each of these resins is molecularly similar, varying only in percent of cross linkage of the polymeric backbones. Dowex 50WX2-400, Dowex 50WX4-400, and Dowex 50WX8-400 have two percent, four percent, and eight percent cross-linkage, respectively. The results in Figure 4 show the recoveries of atrazine, desethyl atrazine, and deisopropyl atrazine from the series of Dowex resins investigated. Two mL of each of the resins were tested for their individual trapping abilities. Recovery was measured on each resin by rinsing with two mL of 0.25 M NaOAc in 50% 2-propanol (IPA). While both Dowex 50WX2-400 and Dowex 50WX4-400 showed similar recoveries, Dowex 50WX8-400 exhibited minimal recovery.

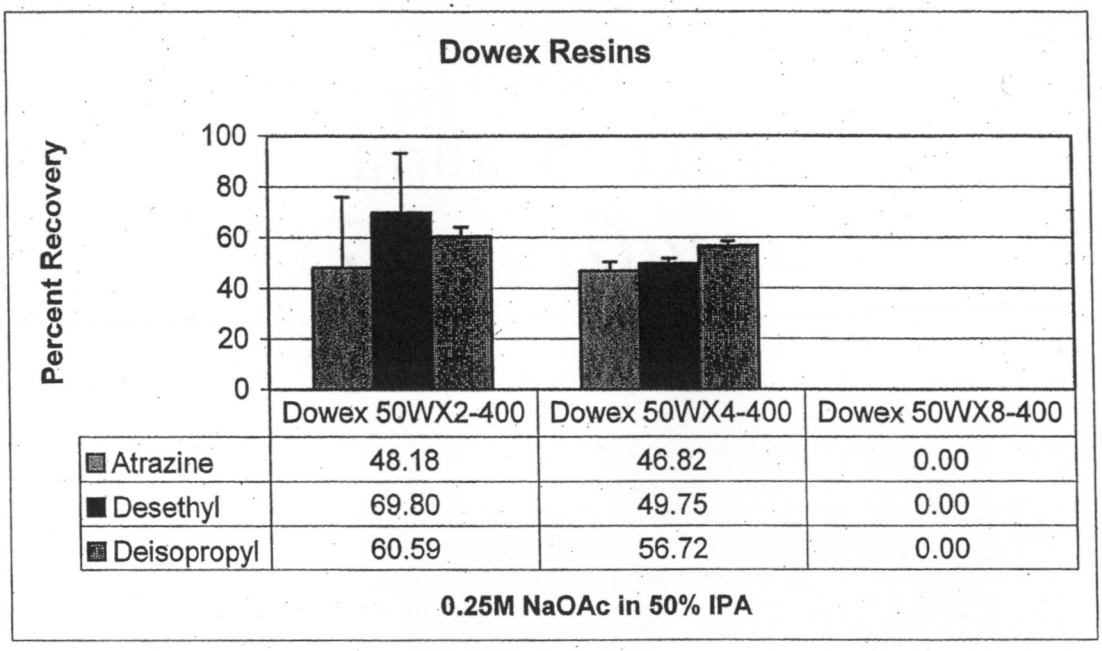
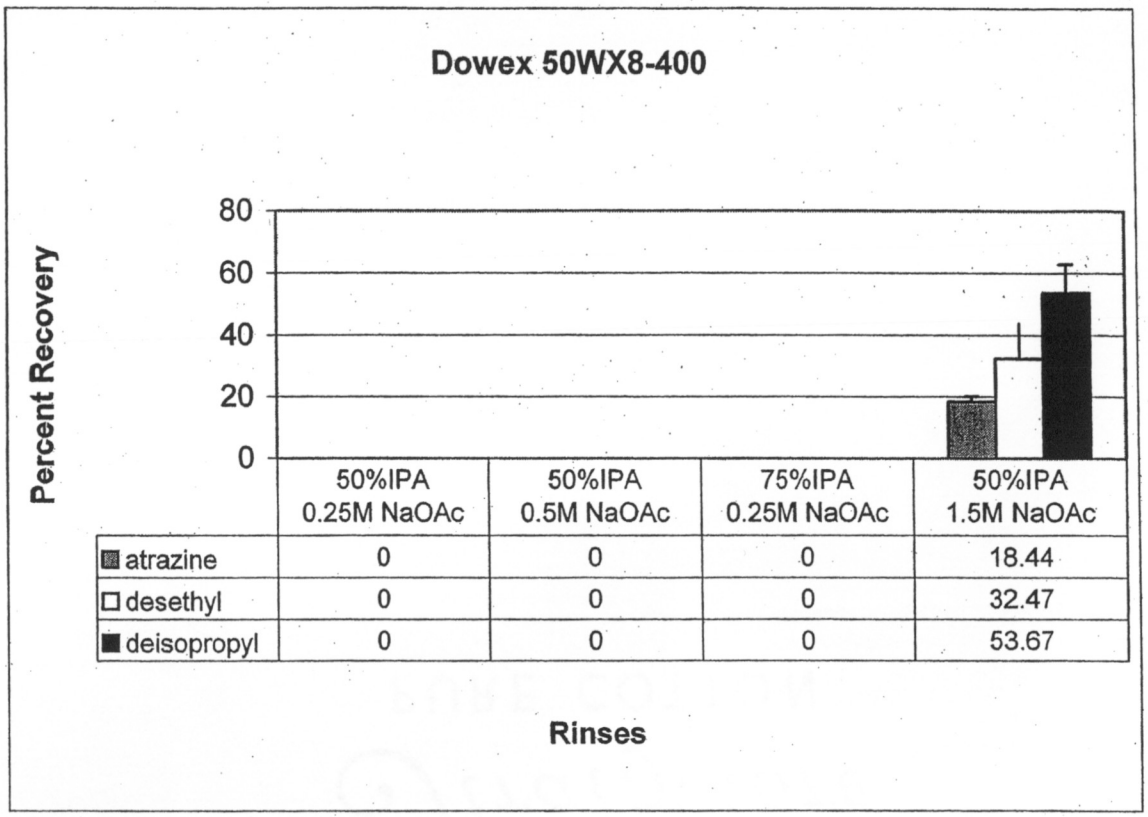


Figure 4. Percent recoveries of atrazine, desethyl atrazine, and deisopropyl atrazine from a series of Dowex resins including: Dowex 50WX2-400, Dowex 50WX4-400and Dowex 50WX8-400. The resin solution used in these experiments was 0.25M NaOAc in 50% IPA

B.1. Dowex 50WX8-400

The low recovery of analytes in the experiments using Dowex 50WX8-400 may be attributed to the higher degree of cross-linkage of the resin. Because there is a great deal of cross-linkage between each polymeric chain of this polystyrene based resin the analytes must diffuse throughout the cross-linked chains. The analytes may be trapped on the resin; however, recovery is difficult. Evidence to support this hypothesis is shown in Figure 5.

A series of experiments was conducted with Dowex 50WX8-400 resin which varied the type of rinse solution and amount of ion-exchanger salt in the rinse solution. Rinse solutions employed included: 0.25 M NaOAc in 50% 2-propanol, 0.50 M NaOAc in 50% 2-propanol, 0.25 M NaOAc in 75% 2-propanol, and 1.5 M NaOAc in 50% 2-propanol. Recovery of analytes was achieved using only 1.5 M NaOAc in 50% 2-propanol as the rinse solution. There was no detectable recovery of any of the metabolites using any of the other above listed rinse solutions. This series of experiments suggests that the metabolites are trapped on the resin; however, in order to successfully remove them from the resin a high concentration of ion exchanger salt is needed.



	50%IPA 0.25M NaOAc	50%IPA 0.5M NaOAc	75%IPA 0.25M NaOAc	50%IPA 1.5M NaOAc
atrazine	0	0	0	18.44
desethyl	0	0	0	32.47
deisopropyl	0	0	0	53.67

Figure 5. Percent recoveries of atrazine, desethyl atrazine, and deisopropyl atrazine from Dowex 50WX8-400 using several different rinse solutions including: 0.25 M NaOAc in 50% 2-propanol, 0.50 M NaOAc in 50% 2-propanol, 0.25 M NaOAc in 75% 2-propanol, and 1.5 M NaOAc in 50% 2-propanol.

B.2. Dowex 50WX2-400

The recovery for Dowex 50WX2-400 was similar to the recovery of Dowex 50WX4-400. Preliminary investigations for this resin focused on developing optimum capture and recovery conditions for the determination of atrazine, desethyl atrazine, deisopropyl atrazine, and didealkyl atrazine. To optimize the procedure, several parameters were tested, including percentage of alcohol in the elution solution and the concentration and type of ion exchanger salt in the elution solution.

B.2.1 Alcohol Concentration

The effect of alcohol concentrations on the elution of atrazine, desethyl atrazine, deisopropyl atrazine, and didealkyl atrazine from Dowex 50WX2-400 using multiple 2-propanol concentrations in the elution solution was measured. 2-propanol serves as an organic modifier to the water based rinse solution. The addition of this organic modifier serves to induce solubility of the analytes in the rinse solution and thereby improve percent recovery. Rinse solutions with 50% and 75% 2-propanol were tested. Each solution was also fortified with 1.0 M NaOAc salt, which serves as the source for ion exchanger ions. Two mL of each rinse solution was pumped through the resin five times. Each rinse was collected individually and the percent recovery of each was calculated for the individual rinses. Figure 6 shows that the rinse solution made of 50% 2-propanol is able to recover a larger amount of analyte than the 75% 2-propanol solution.

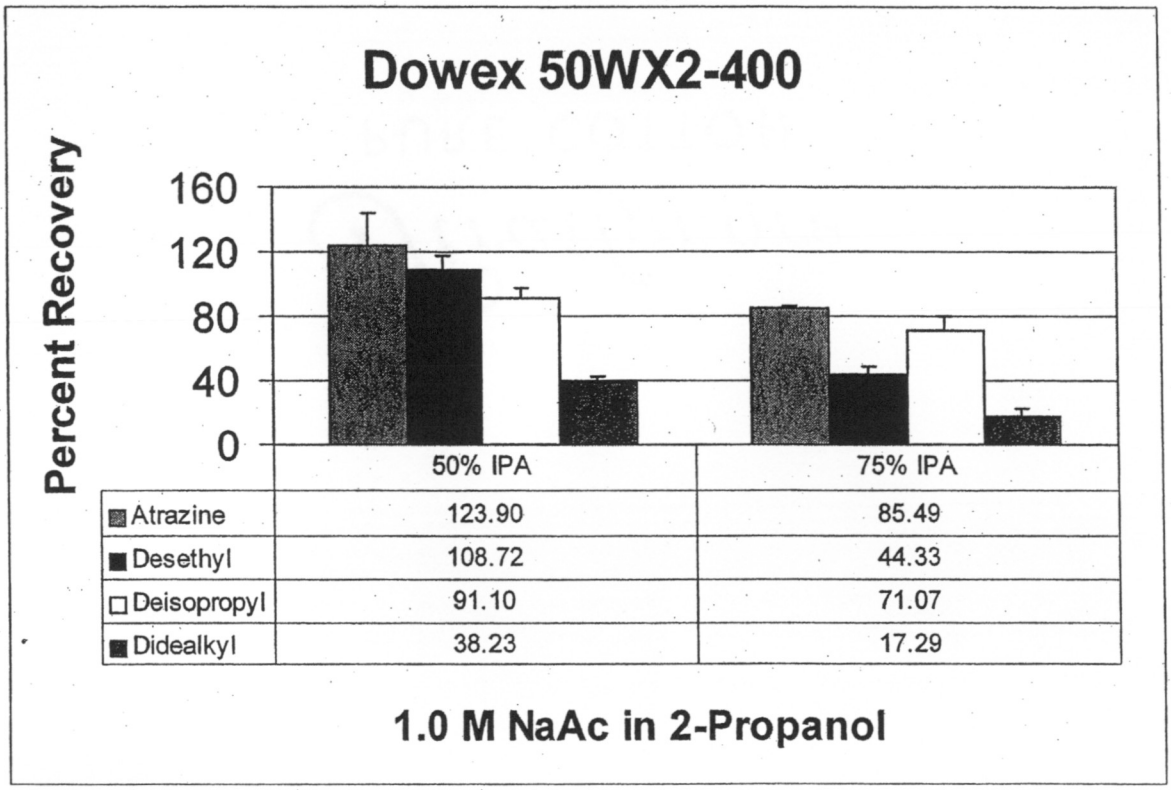


Figure 6. Effect of varying the percentage of IPA in a 1.0M NaOAc rinse solution using Dowex 50WX2-400

B.2.2. Ion Exchanger Salt Concentration

The concentration of ion exchanger salt may have some affect upon the recovery of atrazine, desethyl atrazine, deisopropyl atrazine and didealkyl atrazine. The relationship between salt concentration and analyte recovery was investigated. To explore the effect of ion exchanger salt concentration several elution solutions of varying concentrations of NaOAc were used as rinse solutions. The percent recovery of the analytes was examined. Each rinse solution was two mL. Their concentrations were 0.25 M, 0.50 M, and 1.5 M NaOAc dissolved in 50% 2-propanol. Two mL of each rinse solution was pumped through the resin five times. Each rinse was collected individually, and the percent recovery of each was calculated for the individual rinses. Figure 7 indicates that 0.50 M NaOAc in 50% 2-propanol is the best rinse solution for analyte recovery using Dowex 50WX2-400. This combination gives the highest recovery with very little error. There is also significant recovery of the most polar metabolite, didealkyl atrazine. This metabolite is traditionally difficult to trap using a C_{18} resin.

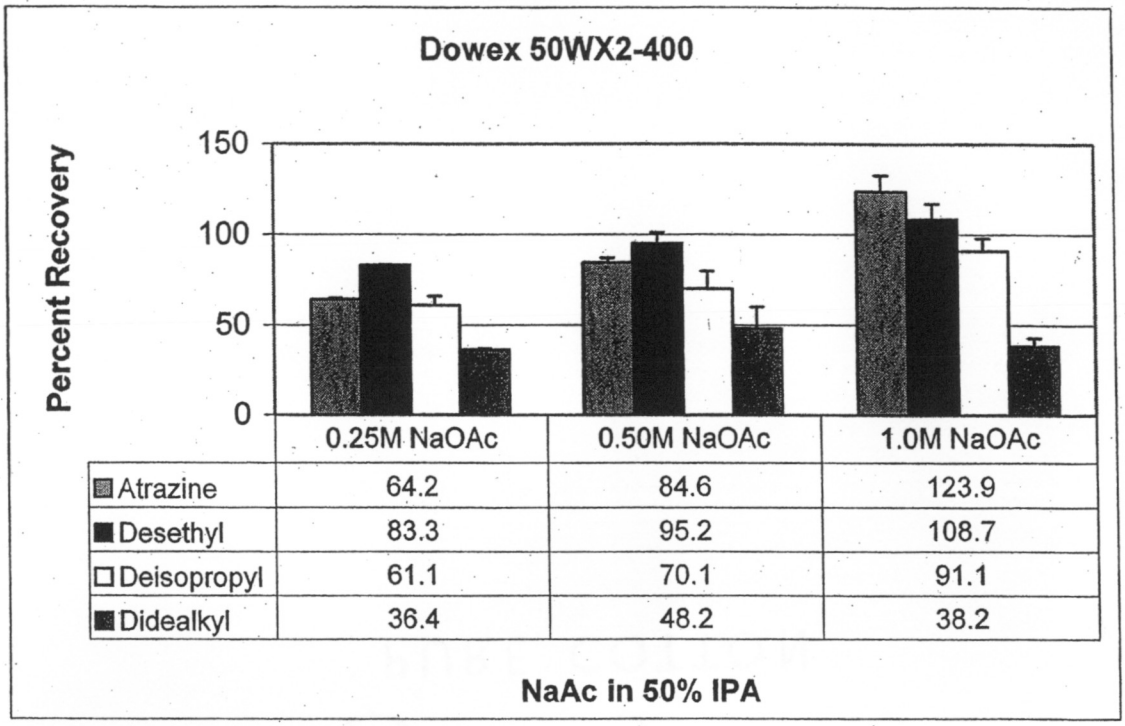


Figure 7. Effect of varying the concentration of NaOAc in a 50% IPA rinse solution using Dowex 50WX2-400

B.3. Dowex 50WX4-400

There was some recovery of each analyte using Dowex 50WX4-400. To determine optimum capture and recovery of atrazine, desethyl atrazine, deisopropyl atrazine, and didealkyl atrazine further experiments were necessary. Optimization parameters included alcohol concentration in the elution solution, type of ion-exchanger salt in the elution solution, concentration of ion-exchanger salt in the elution solution, type of acetate salt in the elution solution, and ionic form of the resin.

B.3.1. Alcohol Concentration

The effect of alcohol concentrations on the elution of atrazine, desethyl atrazine, deisopropyl atrazine, and didealkyl atrazine from Dowex 50WX4-400 resin using multiple 2-propanol rinse concentrations was observed. 2-propanol serves as an organic modifier to the aqueous rinse solution. The rinse solutions contain different concentrations of 2-propanol including: 25%, 50%, and 75%. Two mL of each rinse solution was pumped through the resin five times. Each rinse was collected individually and the percent recovery of each was calculated. Figure 8 shows that there is little dependence of the recovery on the concentration of alcohol in the rinse solution. Each set of experiments shows statistically similar recovery of the analytes, suggesting that recovery is independent of percentage 2-propanol.

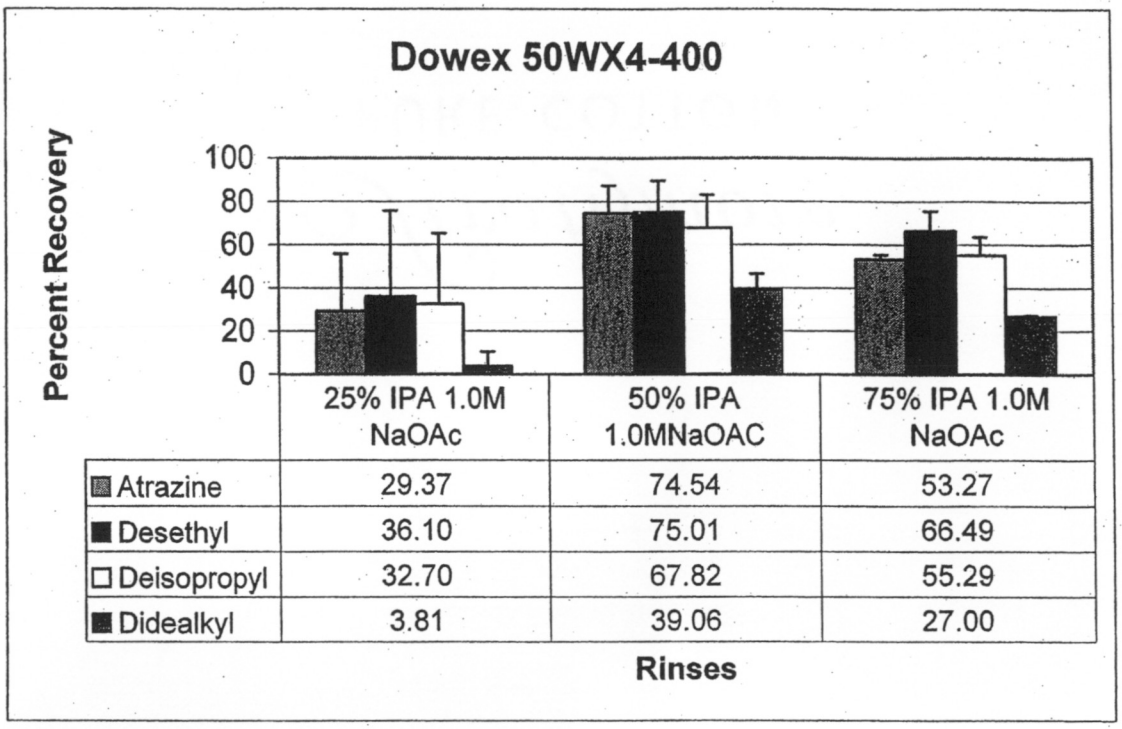


Figure 8. Effect of varying the percent 2-propanol in a 1.0M NaOAc rinse solution using Dowex 50WX4-400

B.3.2. Type of Exchange Ion

The relationship between the type of ion exchange salt used and the percent recovery of metabolites was examined. The exchange ion serves to ultimately release analyte trapped on the resin. The analyte is trapped by extracting hydrogen from the resin resulting in a positively charged analyte that is attracted to the negatively charged resin. Next, an ion exchange salt is introduced to the resin and the anion in the ion exchanger salt forms and acid. The result is the recovery of the analyte and formation of an acid.

To investigate the effect of varying the type of ion exchanger salt two different salts were used including: calcium chloride ($CaCl_2$) and NaOAc. The rinse solutions were 2 mL and their concentrations were 0.25 M of salt dissolved in 50% 2-propanol. Two milliliter aliquots of each rinse solution were pumped through the resin five times. Each rinse was collected individually and the percent recovery of each fraction was calculated for the individual rinses. Figure 9 shows a correlation between the type of ion used and the percent recovery of analytes. Lower recovery of atrazine, desethyl atrazine, and deisopropyl atrazine was observed when calcium chloride was used as the ion exchanger salt. The reason for this may be that a strong acid is generated in the recovery mechanism. As the solution is pumped through the resin, recovery of the analytes is reliant on cation exchange and the generation of an acid. In the experiment using $CaCl_2$ as the ion exchanger salt, a strong acid is produced. When NaOAc is employed as the ion exchanger salt acetic acid is produced, which is a weak acid that highly associates when formed. When introduced to the resin, the acetate group will react with any available hydrogen. It is in this step that the analyte is deprotonated and released from the resin. Using NaOAc yields a dissociated acetate anion that can deprotonate the analyte more

easily than the chloride ion. Also, the formation of acetic acid when NaOAc is used as the rinse solution is beneficial to the life of the Microsorb C_{18} column in the HPLC as regards maintaining a pH above 3. The column cannot accomodate samples of extreme pHs.

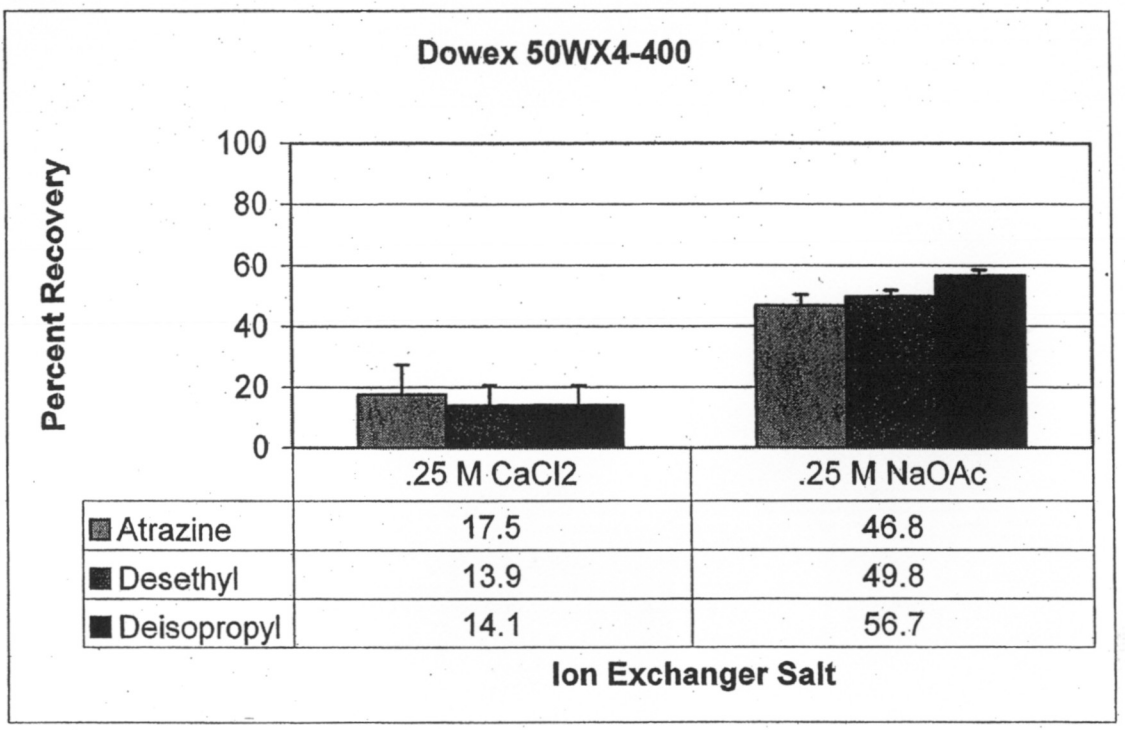
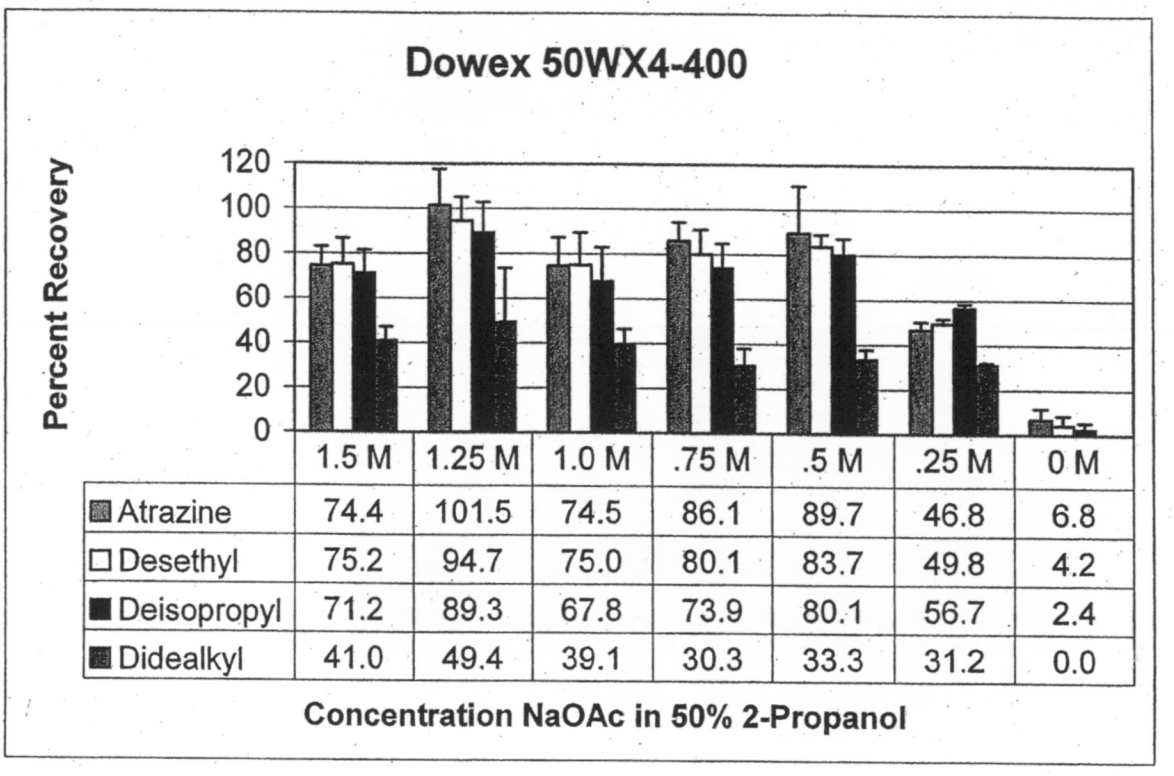


Figure 9. Effect of type of ion exchanger salt on analyte recovery using Dowex 50WX4-400

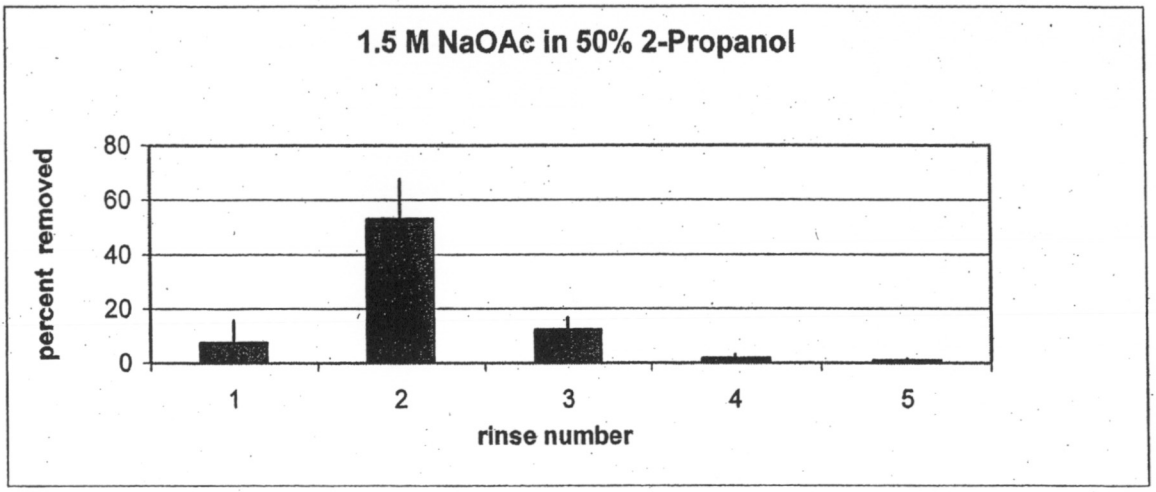
B.3.3. Ion Exchanger Concentration

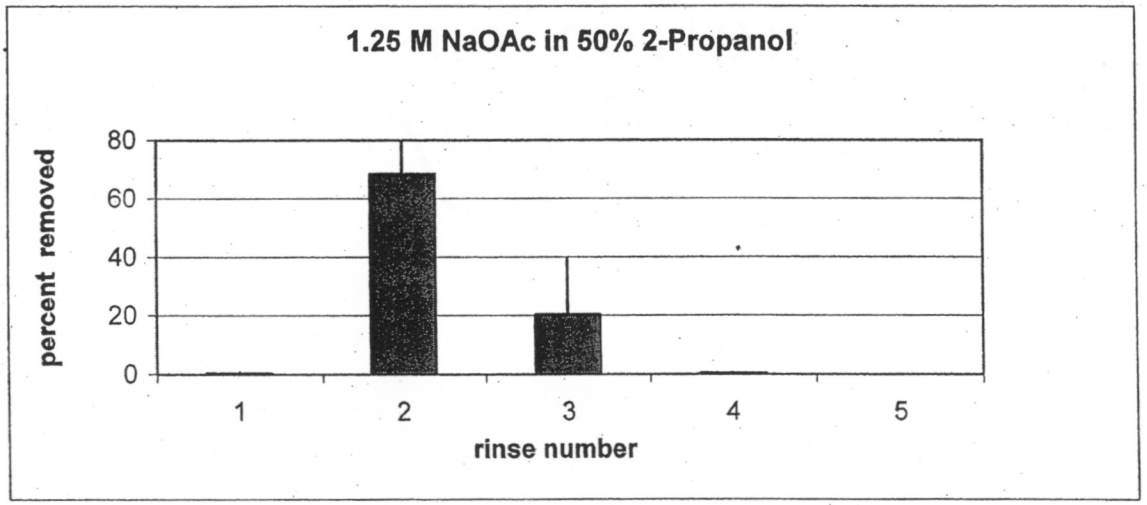
The concentration of ion exchanger salt may have some affect upon the recovery of atrazine, desethyl atrazine, deisopropyl atrazine and didealkyl atrazine. The relationship between salt concentration and analyte recovery was investigated. To investigate the effect of ion exchanger salt concentration, elution solutions of varying concentrations of NaOAc were used as rinse solutions. The percent recovery of the analytes was determined. Each rinse solution was two mL, with varying concentrations of NaOAc including: 0 M, 0.25 M, 0.50 M, 0.75 M, 1.0 M, 1.25 M, and 1.5 M NaOAc dissolved in 50% 2-propanol. Two mL of each rinse solution was pumped through the resin five times. Percent recovery of each fraction was calculated for the individual rinses. Figure 10 shows that the presence of NaOAc is necessary for recovery of atrazine, desethyl atrazine, deisopropyl atrazine and didealkyl atrazine. The correlation between analyte recovery and NaOAc is more pronounced upon inspection of the rinse profiles for each experiment. The profiles show that those rinses with a higher concentration of NaOAc allow elution of analytes more rapidly, while lower concentrations of NaOAc achieve a comparable percent recovery in later rinses. This early elution in higher salt concentration rinses is due to the greater availability of exchanger ions to perform cation exchange in earlier rinses thereby releasing the analytes in earlier rinses. Using a higher concentration of salt reduces the amount of sample manipulation.



	1.5 M	1.25 M	1.0 M	.75 M	.5 M	.25 M	0 M
Atrazine	74.4	101.5	74.5	86.1	89.7	46.8	6.8
Desethyl	75.2	94.7	75.0	80.1	83.7	49.8	4.2
Deisopropyl	71.2	89.3	67.8	73.9	80.1	56.7	2.4
Didealkyl	41.0	49.4	39.1	30.3	33.3	31.2	0.0

Figure 10. Effect of salt concentration on recovery of analytes


1.5 M NaOAc in 50% 2-Propanol
percent removed
80
60
40
20
0
1
2
3
4
5
rinse number


1.25 M NaOAc in 50% 2-Propanol
percent removed
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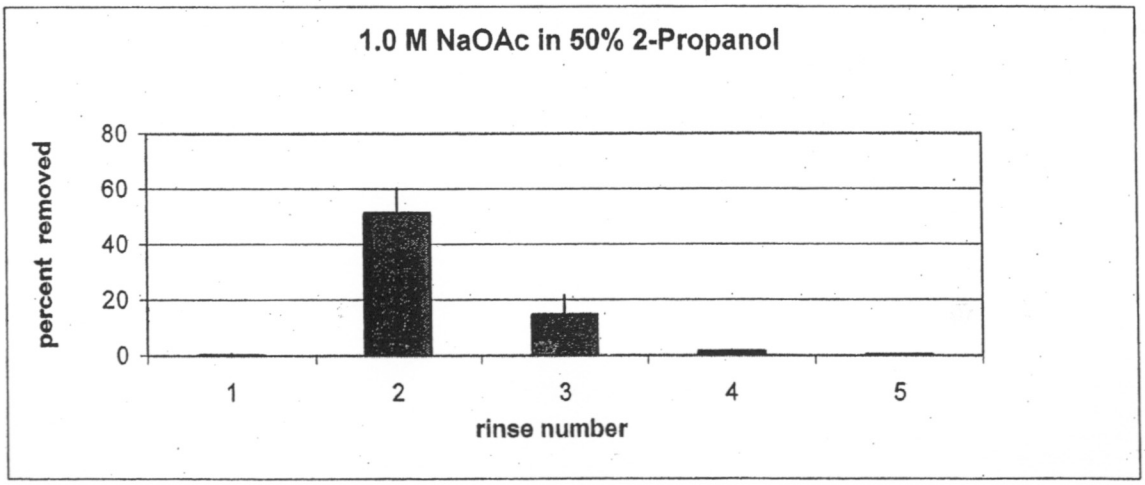
rinse number


1.0 M NaOAc in 50% 2-Propanol
percent removed
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60
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20
0
1
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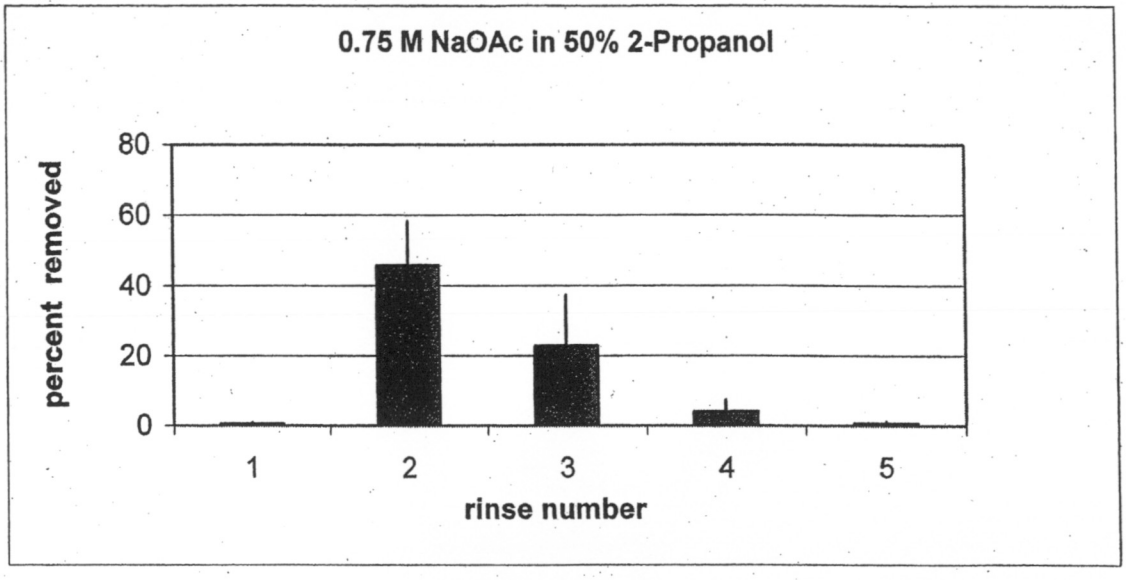
3
4
5
rinse number


0.75 M NaOAc in 50% 2-Propanol
percent removed
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60
40
20
0
1
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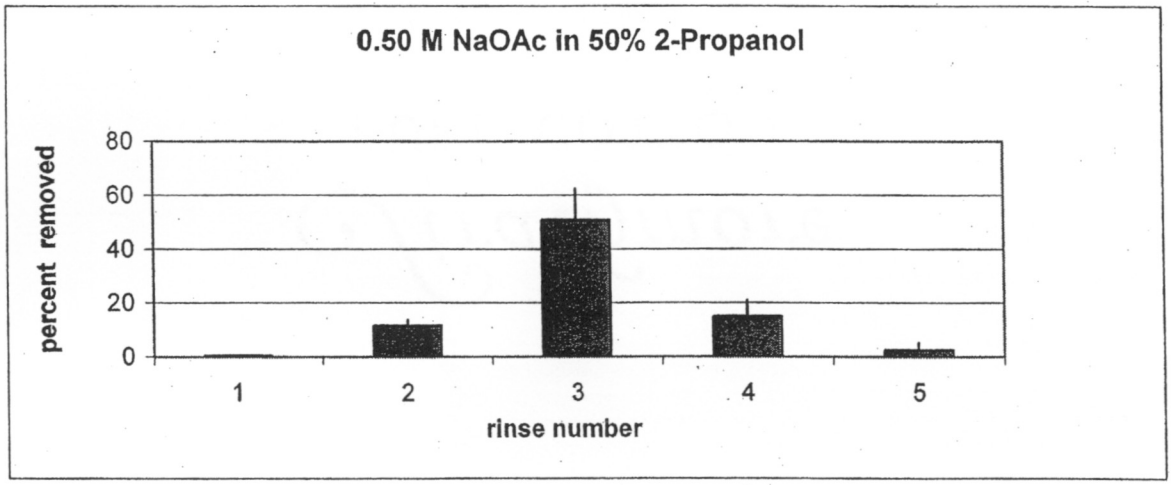
5
rinse number


0.50 M NaOAc in 50% 2-Propanol
percent removed
80
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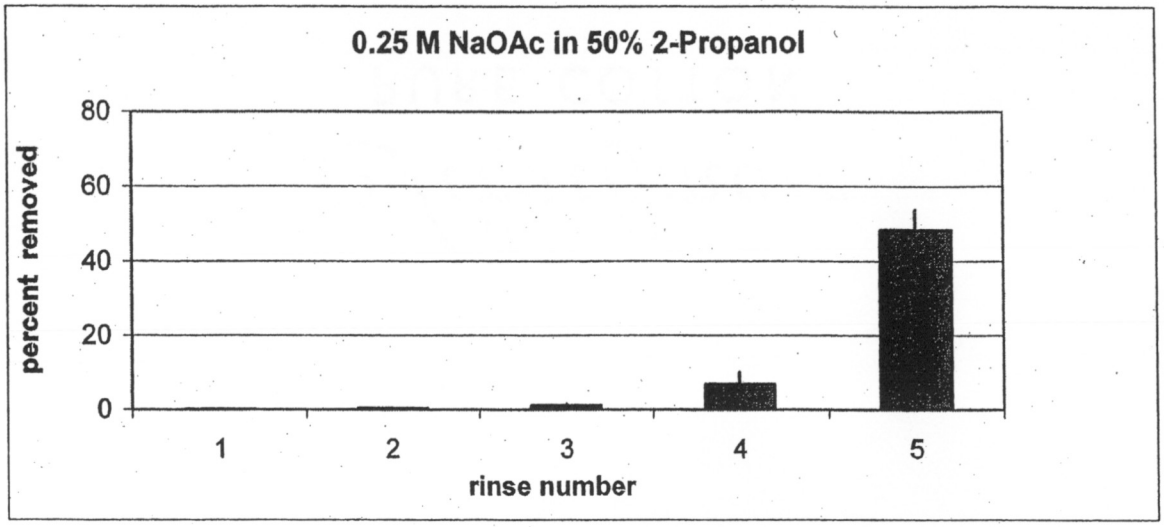
rinse number



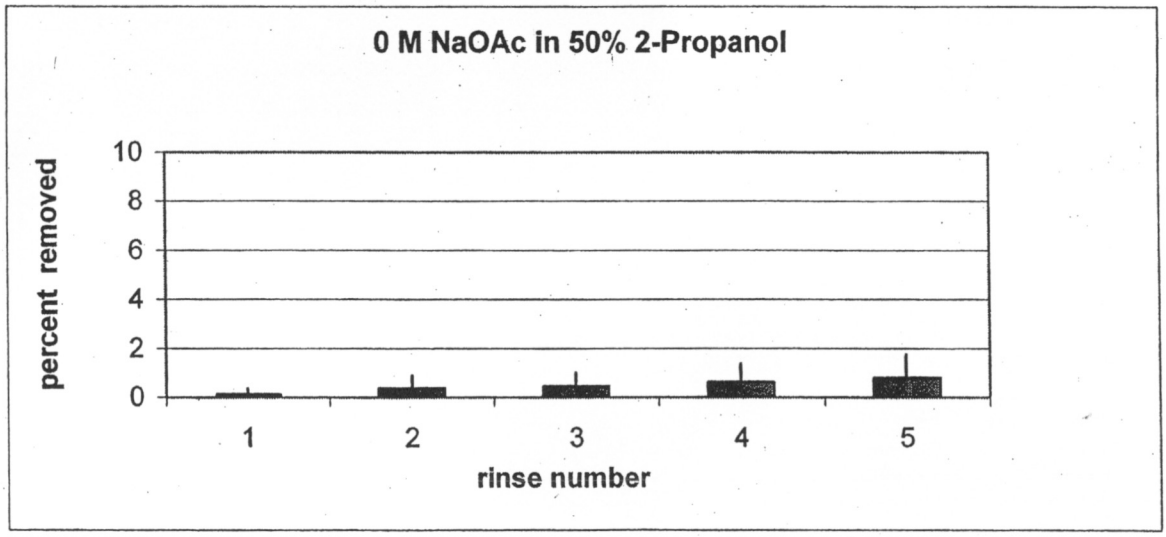
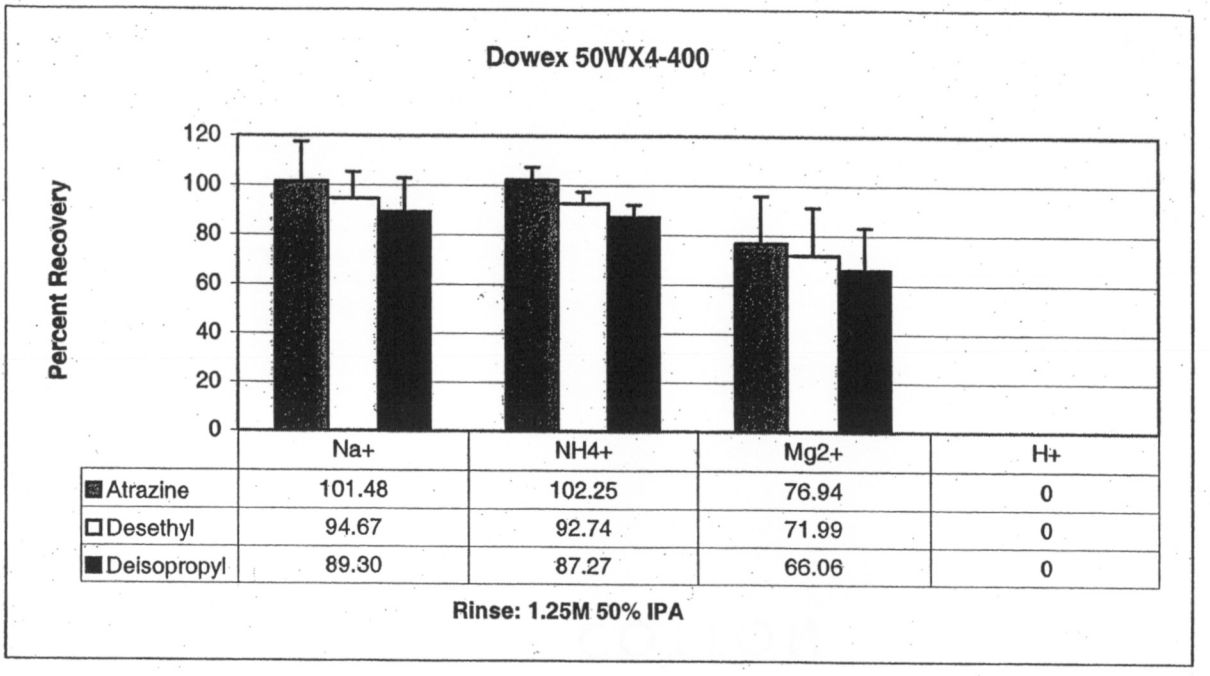


Figure 11. Rinse profiles. This shows the effect of decreasing the concentration of ion-exchanger salt in the rinse solution. The analytes are eluted in later rinses at lower salt concentration.

B.3.4. Type of Acetate Elution Solution

To further investigate the role of the ion exchanger salt in the elution solution a set of experiments was performed to monitor the effects of different types of acetate salts. Experiments were performed to determine whether analyte recovery was dependent upon cation exchange between sodium and the resin, or the formation of acetic acid. To observe this relationship a set of experiments incorporating different kinds of acetate salts in the elution solution was performed. Each elution solution was two mL. The acetate salts included in the rinse solutions were: NaOAc, ammonium acetate (NH_4OAc), magnesium acetate ($MgOAc_2$), and acetic acid (HOAc). Each salt had a charge concentration of 1.25 M, and was dissolved in 50% 2-propanol. Figure 12 shows that the type of acetate salt used has no impact upon analyte recovery. The experiment including acetic acid as the rinse solution resulted in no recovery of any of the analytes. Using this weak acid as the rinse solution did not allow ion exchange because of the high degree of association of the hydrogen and acetate ions in acetic acid. Each of the recoveries using the other forms of acetate was statistically similar. The indication is that ion-exchange is an important step in analyte recovery.



	Na+	NH4+	Mg2+	H+
Atrazine	101.48	102.25	76.94	0
Desethyl	94.67	92.74	71.99	0
Deisopropyl	89.30	87.27	66.06	0

Figure 12. Effect of different types of acetate salts in a 1.25M charge concentration in a 50% IPA rinse solution using Dowex 50WX4-400

B.3.5. Ionic Form of Dowex 50WX4-400

Dowex 50WX4-400 is initially in a protonated form. To determine the importance and role of the hydrogen on the resin for the extraction of atrazine and metabolites, Dowex 50WX4-400 was tested in different ionic forms. The percent recovery of analyte was calculated for each of the ionic forms of the resin. The rinse solution used for each experiment was 1.25 M NaOAc in 50% 2-propanol. The ionic forms of the resin tested and compared were H^{1+}, NH_4^{1+}, and Ca^{2+}. Figure 13 shows that the ionic form of the resin has an effect on analyte recovery. It is absolutely necessary that the resin be in the hydrogen form. This form allows protonation of the primary and secondary amines on each of the analytes, allowing sufficient capture. This acid-base interaction is necessary for analyte trapping. When the resin is in a different ionic form, a strong analyte-sorbent interaction does not exist to retain these analytes.

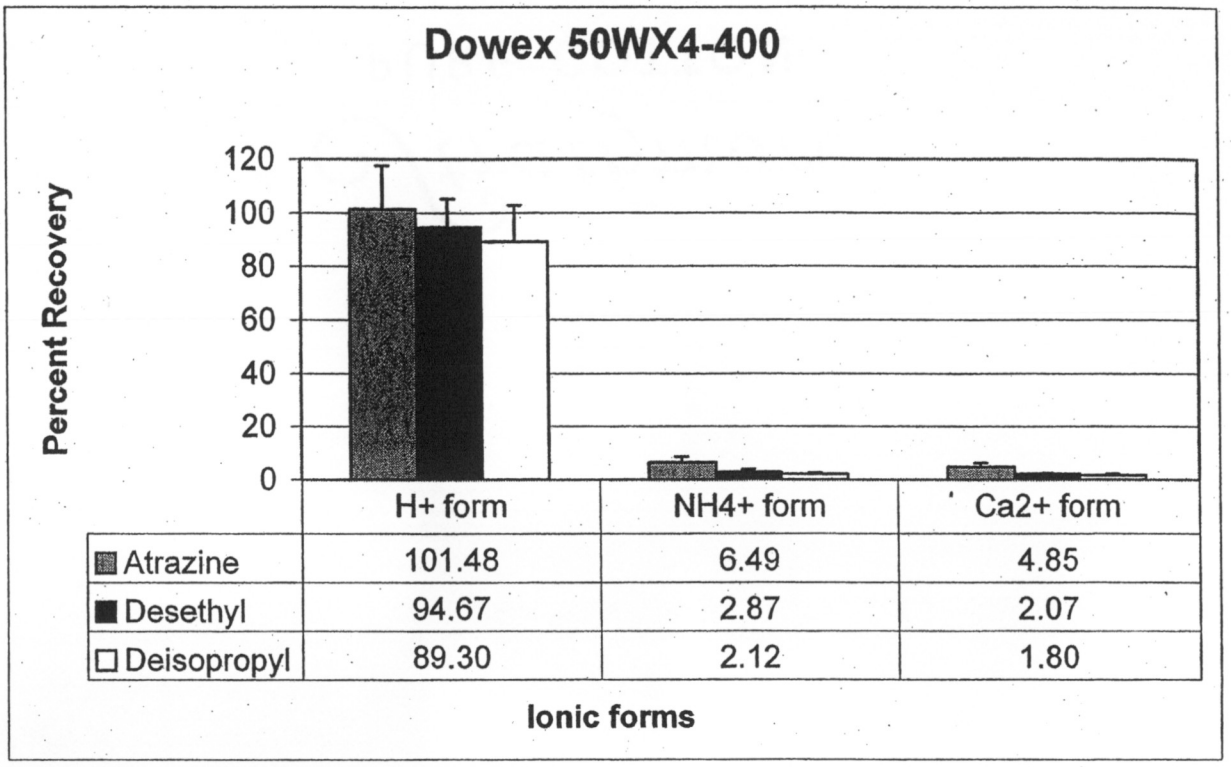
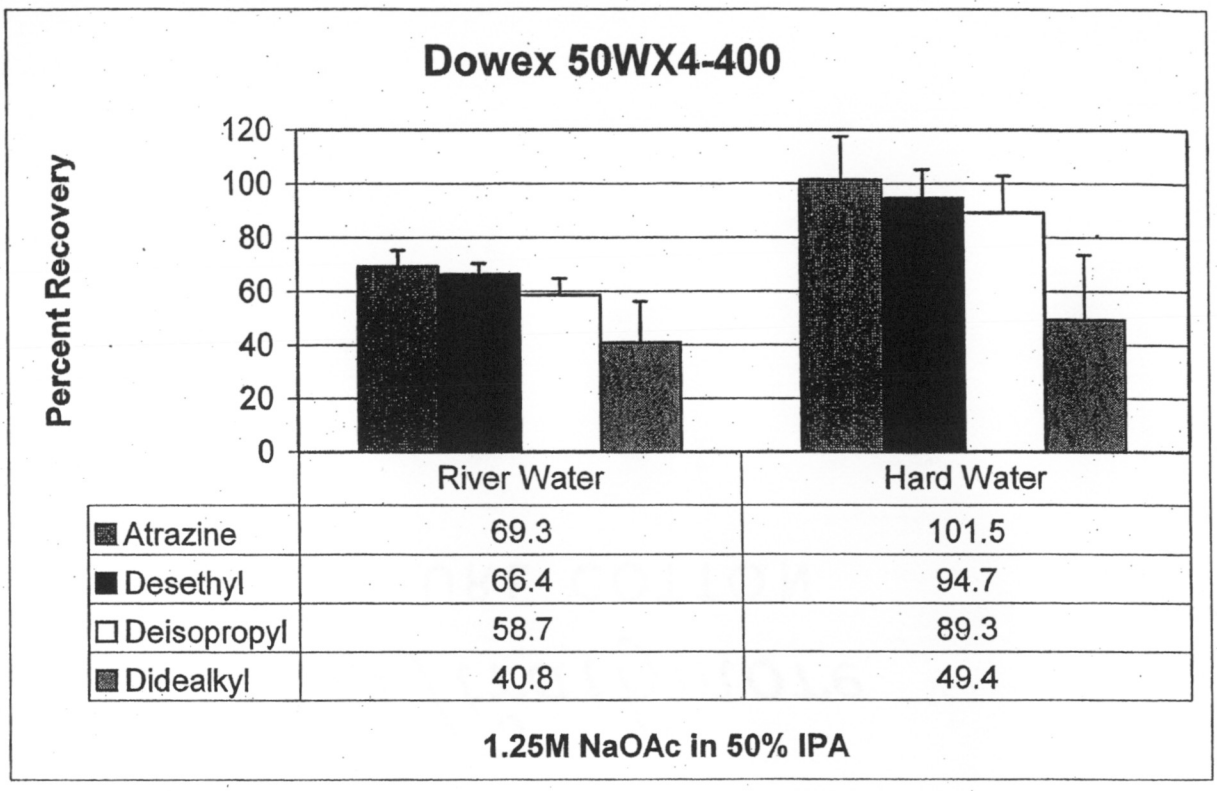


Figure 13. Effect of varying the ionic form of the resin for capture and recovery of the analytes.

C. River Water Sample

All of the experiments that have been done have been performed using 500 mL of deionized water fortified with 500 μL of 1 M $CaCl_2$ to imitate a hard water matrix. It is important to test the determination of atrazine, desethyl atrazine, deisopropyl atrazine, and didealkyl atrazine in a real water sample because the analytes are primarily found in rivers, lakes, streams, groundwater, and any other water exposed to the environment. A water sample was taken from the Barren River in Kentucky. An experiment was conducted using the 500 mL of river water spiked with 500 μL of the atrazine cocktail. Figure 14 compares this experiment to one done with 500 mL of deionized water fortified with 500 μL of 1 M $CaCl_2$ and 500 μL of the atrazine cocktail. In both experiments Dowex 50WX4-400 was used as the resin, with a rinse solution of 1.25 M NaOAc. The results show a difference in percent recovery of some of the analytes when using river water and simulated hard water.



	River Water	Hard Water
Atrazine	69.3	101.5
Desethyl	66.4	94.7
Deisopropyl	58.7	89.3
Didealkyl	40.8	49.4

Figure 14. Effect of using river water versus using a simulated hard water matrix as the sample.

D. Limits of Detection and Quantitation

Because of the great toxicity of these compounds and the documented adverse health effects, the U.S. Environmental Protection Agency (EPA) has set the maximum contamination level (MCL) for atrazine at three parts per billion.[24] It is essential to detect these compounds near the MCL to make this procedure viable. To determine the limits of detection and quantitation a calibration curve was plotted using stock solutions with various analyte concentrations. Using this calibration curve the limits of detection and quantitation were calculated. The calculations showed the detection limits were: 0.81 ppb, 0.70 ppb, 0.70 ppb, and 1.24 ppb for atrazine, desethyl atrazine, deisopropyl atrazine, and didealkyl atrazine, respectively. The limits of quantitation were 1.19 ppb, 1.08 ppb, 1.04 ppb, and 1.71 ppb, respectively. The procedure is certainly viable for the determination of atrazine and its metabolites with regards to the limits of detection and quantitation.

IV. CONCLUSIONS

Based upon the data presented in this study Dowex 50WX4-400 offers good recovery of atrazine, desethylatrazine, deisopropylatrazine, and didealkylatrazine from a hard water matrix. Using this SPE procedure this sorbent can extract atrazine and selected metabolites.

A SPE procedure incorporating Dowex 50WX4-400 resin and using a rinse solution of 1.25M NaOAc in 50% IPA could trap and recover atrazine, desethyl atrazine, deisopropyl atrazine, and didealkyl atrazine in aqueous samples. Recovery of these the more polar metabolites, deisopropyl atrazine, and didealkyl atrazine, using Dowex 50WX4-400 was greater than traditionally used silica based C_{18} resin,

The resin must be in hydrogen form to trap the analytes. The analytes are trapped through becoming protonated and then attracted to the resin through an acid-base interaction. Without the presence of hydrogen, this interaction is not possible and trapping cannot occur.

Ion-Exchange salts are required for analyte recovery. These salts must include ions that are dissociated when dissolved in the rinse solution. The cations must be available for cation exchange with the resin, allowing the release of the analyte. It is also beneficial for the anion of the salt to deprotonate the analyte to form a weak acid. The formation of this weak acid is important to maintain a moderate pH. A neutral pH is desirable to protect the integrity of the Microsorb C_{18} column.

Rigorous contaminant limits for water purity that have been set by the U.S. EPA require analyte determination procedures that can achieve extremely low levels of detection. Currently, the U.S. EPA has set the MCL for atrazine at three ppb.[24] Using this

SPE procedure as a preconcentration step for analyte determination by RP-HPLC-PDA, we are able to detect atrazine, desethyl atrazine, deisopropyl atrazine, and didealkyl atrazine in aqueous samples below the MCL.

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